Epidemiologic determinants of vaginal pH

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OBJECTIVES: This study was undertaken to evaluate the relationship between vaginal pH and factors related to cervical cancer.

STUDY DESIGN: In a population-based sample of 9161 women from Guanacaste Province in Costa Rica women were categorized into 2 groups, those with vaginal pH in the reference range (4.0-4.5) and those with elevated vaginal pH (5.0-5.5). Odds ratios were used to estimate the relationship between elevated pH and its potential determinants.

RESULTS: Aging was strongly associated with increasing vaginal pH, starting at around 45 years of age and continuing into old age. Menopause was responsible for an additional 1.7-fold increase in the odds of having an elevated pH (odds ratio 1.7, 95% confidence interval 1.4-2.0). Human papillomavirus infection and cervical intraepithelial neoplasia were not associated with changes in pH.

CONCLUSIONS: Our data indicate that vaginal pH is strongly related to age and to menopausal status and thus could be a marker of age-related hormonal changes. Elevated pH does not appear to be associated with risk of high-grade intraepithelial neoplasia among women infected with human papillomavirus. (Am J Obstet Gynecol 1999;180:1060-6.)

Key words: Aging, bacterial vaginosis, cervical neoplasia, menopause, vaginal pH

A vaginal pH between 4.0 and 5.0 is considered normal for women with active menstrual cycles.[1] Among premenopausal women, ovarian hormones facilitate vaginal colonization with Döderlein lactobacilli, which produce lactic acid from vaginal glycogen, thus maintaining vaginal acidity.[1, 2] After menopause the pH increases, probably as a result of the decline in circulating estrogens that causes a gradual loss of glycogen and lactobacilli.[2, 3] Changes in vaginal pH have also been observed in premenopausal women during the menstrual cycle[1, 3] and in postmenopausal women after administration of estrogen,[4-7] further indicating that vaginal pH is closely related to ovarian hormones. Alterations of the vaginal microflora not directly related to ovarian function, such as bacterial vaginosis and vaginal infections, are also usually accompanied by changes in pH.[2, 7] Finally, such other factors as aging,[2] sexual activity,[8, 9] and vaginal douching[2] may affect vaginal pH; however, relative contributions of these factors are unclear.

Because vaginal pH results from the interplay of many factors affecting the vaginal microenvironment and can be easily and inexpensively measured, it could be a useful marker of the vaginal microenvironment in epidemiologic studies or in clinical practice. A marker of the vaginal microenvironment would be particularly useful in epidemiologic studies of cervical carcinogenesis because factors that can alter the vaginal microenvironment, such as vaginal hygiene practices,[10] bacterial vaginosis,[11] and sexually transmitted infections,[12] have been proposed to play a role as cofactors for persistence of human papillomavirus (HPV) infection, the main etiologic factor in cervical carcinogenesis. A better understanding of the factors affecting vaginal pH might enhance our ability to study its possible importance.

In a large population-based study of the natural history of HPV infection and cervical neoplasia conducted in Guanacaste Province, Costa Rica, we evaluated the relationship between vaginal pH and factors related to cervical cancer, such as age, smoking history, reproductive history, sexual habits, history of vaginal infections, HPV

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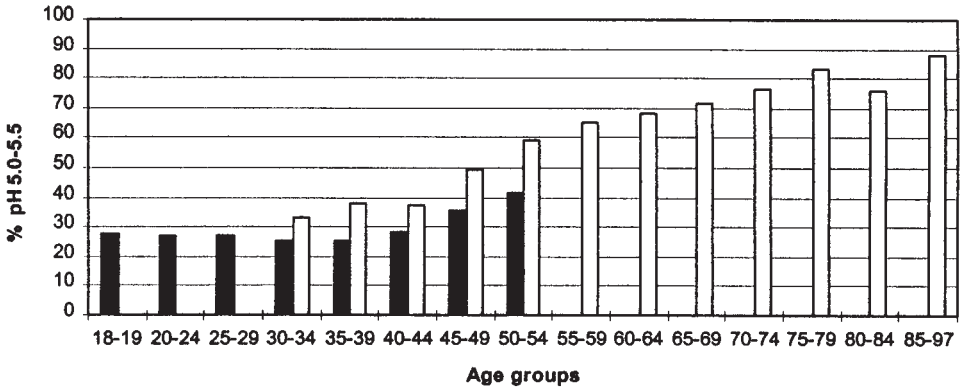

Fig 1. Percentage of women with vaginal pH between 5.0 and 5.5 according to age group (in years) and menopausal status among 9161 women from Guanacaste Province, Costa Rica. *Filled bars,* Premenopausal women; *open bars,* postmenopausal women.

infection, and cervical intraepithelial neoplasia. To our knowledge this is the first study that has evaluated the influences on vaginal pH in a large population-based sample of adult women.

Material and methods

Study population. Potential determinants of vaginal pH were examined during the enrollment phase of a population-based study of cervical neoplasia in Guanacaste, a rural province of Costa Rica. The overall methodology of this study has been described previously in detail.[13] Briefly, a sample of about a sixth of the 1038 Guanacastecan census segments was randomly selected. In the selected segments all women ≥18 years old were identified and invited to participate. Of the 11,742 women identified from the selected census segments, 2.6% were excluded from the study because they were pregnant when contacted and would not have completed 3 postpartum months for a reappointment before the enrollment phase ended; 1.6% were excluded because they had mental or language problems, were physically incapacitated, or had died; and 4.4% were excluded because they had moved out of the area. This left a total of 10,738 eligible women (91.4%). Of these eligible women 10,049 (93.6%) agreed to participate and were interviewed after giving informed consent. A validated personal interview included questions on socioeconomic, demographic, sexual, reproductive, medical, and smoking histories. A pelvic examination was offered to women who reported previous sexual intercourse in the interview. Among the 9466 women eligible for a pelvic examination 9175 (96.9%) had the pelvic examination performed, and pH measurements were successfully obtained from 9161 (99.8%) of these women. Menopausal status was defined according to self-report at the personal interview of "still having menstrual periods." This interview was obtained at the same examination when pH measurements were performed. Approval for human experimentation was obtained from both Costa Rican and US institutional review boards.

Vaginal pH measurements. Vaginal pH was measured with a pHydrion strip (Micro Essential Laboratories, Brooklyn, NY), which measures pH across a range of 3.0 to 5.5 in increments of 0.5 pH unit. After insertion of an unlubricated sterile speculum the pHydrion strip was placed on the lateral vaginal wall between the speculum blades until it became wet. Color change of the strip was immediately compared with the colorimetric scale and the measurement was recorded.

Classification of cervical lesions. Cervical lesions were classified as cancer, high-grade squamous intraepithelial lesions, low-grade squamous intraepithelial lesions, or equivocal. For the final diagnosis a combination of cytologic examination (conventional Papanicolaou smear, PapNet Video Images, Neuromedical Systems Inc, Suffern, NY, and ThinPreps, Cytyc Corp, Boxborough, Mass) and samples obtained through colposcopically directed biopsy were reviewed by a team of experts in Costa Rica and the United States.[13] Difficult diagnoses were reviewed and adjudicated by the lead study pathologist (M.E.S.). All cervical cancers, 91.5% of high-grade squamous intraepithelial lesions, but fewer low-grade squamous intraepithelial lesions were histologically confirmed. Presence of deoxyribonucleic acid from 11 cancer-associated HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, and 58) was assessed with Hybrid Capture (Digene Diagnostics, Silver Spring, Md), a Food and Drug Administration–approved kit.[13]

Statistical analysis. Women were dichotomized into a group with vaginal pH values of 4.0 to 4.5 and a second group with values of 5.0 to 5.5. The cutoff between 4.5 and 5.0 was chosen because reference pH values among premenopausal women range between 4.0 and 5.0[2] and a pH value >4.5 is used in the clinical diagnosis of bacterial

Table I. Distribution of vaginal pH values according to menopausal status

	Premenopausal		*Postmenopausal*	
pH	*No.*	*%*	*No.*	*%*
4.0	300	4.8	31	1.1
4.5	4236	67.6	1003	34.6
5.0	1319	21.1	1079	37.2
5.5	409	6.5	784	27.1
TOTAL	6264	100	2897	100

vaginosis.[14] We also compared women with pH values of 4.0 to 4.5 separately against women with pH values of 5.0 and women with pH values of 5.5.

The distribution of vaginal pH was compared between women with different characteristics. Odds ratios and 95% confidence intervals estimated from logistic regression models were used as a measure of association between vaginal pH and its potential determinants. For each characteristic odds ratios were defined as the ratio of the odds of having an elevated pH among women with the characteristic to the odds among women without the characteristic. Age-adjusted models and models adjusted for both age and other factors gave similar estimates of odds ratios. For simplicity of presentation we only show the latter estimates.

Results

The 9161 women included in the study had a mean age of 41 ± 15 years (range 18-94 years). Of these women 6264 (68%) reported still having menstrual periods and 2897 (32%) reported not having menstrual periods when vaginal pH measurements were obtained. In the rest of this article women who reported still having and not having menstrual periods are referred to as *premenopausal* and *postmenopausal*, respectively. Among the postmenopausal women only 77 were <40 years old and were thus unlikely to have had a natural menopause. Most of these women (66%) reported having undergone a hysterectomy, with removal of the ovaries likely.

Table I shows the distribution of vaginal pH values stratified by menopausal status. No pH readings of 3.0 or 3.5 were reported for premenopausal or postmenopausal women. Most premenopausal women had pH readings of 4.5 (67.6%), whereas postmenopausal women were more evenly distributed across pH categories of 4.5 (34.6%), 5.0 (37.2%), and 5.5 (27.1%).

We observed an increase in vaginal pH with increasing age in both premenopausal and postmenopausal women (Fig 1). The percentage of women with a pH of 5.0 to 5.5 was about 26% among menstruating women <40 years old; it started to gradually increase until it reached about 40% among premenopausal women 50 to 54 years of age. On the other hand, among postmenopausal women we observed a continuous increase in the percentage of women with vaginal pH of 5.0 to 5.5 from about 33% among women 30 to 34 years of age until it reached 90% among women ≥85 years old. Finally, among women within the same age group a higher percentage of postmenopausal women than premenopausal women had pH values of 5.0 to 5.5, indicating that the absence of menstrual periods has an impact on vaginal pH independently of age.

Table II presents the distributions of vaginal pH and the estimated odds ratios for variables that presented significant associations with vaginal pH and were measured among women both with and without menstrual periods. Tables III and IV present the pH distributions and odds ratios for variables measured only among premenopausal and postmenopausal women, respectively. Table II shows how the odds of having a vaginal pH of 5.0 to 5.5 increases starting at about 45 years of age and that postmenopausal women had 70% greater odds of having a vaginal pH of 5.0 to 5.5 than did premenopausal women. Single and widowed women had 50% and 20% increases, respectively, in the odds of having a pH of 5.0 to 5.5 with respect to married women. Overall, women who were sexually active in the previous year had a 10% decrease in the odds of having a pH of 5.0 to 5.5 with respect to sexually inactive women. This association was present among postmenopausal women (odds ratio 0.7, 95% confidence interval 0.6-0.9) but not among premenopausal women (odds ratio 1.0, 95% confidence interval 0.8-1.1; test for interaction, $P = .006$). Finally, women with a history of yeast infection had a 10% decrease in the odds of having a pH of 5.0 to 5.5 with respect to women without such a history. Level of education, smoking status, age at menarche, age at first pregnancy, number of live births, lifetime number of sexual partners, frequency of acts of intercourse in the previous year, number of sexual partners in the previous year, and history of venereal infections were not significantly associated with vaginal pH.

According to Table III, compared with women in the secretory phase of the menstrual cycle those who were menstruating (days 1-5), were in the proliferative phase, or had delays in menstrual periods had an increased vaginal pH. The increase in pH was especially strong during menstruation. Premenopausal women who had used barrier contraceptive methods in the previous month had a 20% decrease in the odds of having a pH of 5.0 to 5.5 with respect to women not using barrier methods, and women using intrauterine contraceptive devices had a 40% increase in the odds of having a pH of 5.0 to 5.5. Recent use of oral contraceptives was not significantly associated with vaginal pH. Among postmenopausal women, after taking the effect of age into account, increasing time since last menstrual period was associated with increasing odds of having a pH of 5.0 to 5.5, whereas

Table II. Adjusted odds ratios for association between vaginal pH from 5.0 to 5.5 and selected variables among premenopausal and postmenopausal women

	Vaginal pH 4.0-4.5		*Vaginal pH 5.0-5.5*			
	No.	*%*	*No.*	*%*	*Odds ratio**	*95% confidence interval*
Age group						
<25 y	756	72.97	280	27.03	1.0	—†
25-29 y	921	72.98	341	27.02	1.0	0.9-1.3
30-34 y	992	74.70	336	25.30	1.0	0.8-1.1
35-39 y	888	74.56	303	25.44	0.9	0.8-1.1
40-44 y	693	70.79	286	29.21	1.1	0.9-1.3
45-49 y	466	59.90	312	40.10	1.5	1.2-1.9
50-54 y	261	43.43	340	56.57	2.1	1.6-2.8
55-59 y	183	35.40	334	64.60	2.7	2.0-3.6
60-64 y	140	31.89	299	68.11	3.0	2.2-4.1
65-69 y	107	28.76	265	71.24	3.5	2.5-4.9
70-74 y	62	23.22	205	76.78	4.5	3.1-6.5
75-79 y	25	17.61	117	82.39	6.1	3.7-10.1
≥80 y	28	20.29	110	79.71	4.9	3.0-8.0
Menopausal status						
With menstrual cycles	4500	72.7	1686	27.3	1.0	—†
Without menstrual cycles	1022	35.7	1842	64.3	1.7	1.4-2.1
Marital status						
Married	4631	65.3	2456	34.7	1.0	—†
Divorced	261	51.4	247	48.6	1.0	0.8-1.2
Widowed	135	29.3	326	70.7	1.2	1.0-1.6
Single	495	49.8	499	50.2	1.5	1.3-1.8
Sexually active in previous year‡						
No	1616	46.8	1841	53.2	1.0	—†
Yes	3903	69.5	1687	30.5	0.9	0.8-1.0
History of yeast infection						
No	3377	57.7	2472	42.3	1.0	—†
Yes	2145	67.0	1056	32.0	0.9	0.8-0.9

*Derived from complete data on 9050 women.
†Reference category; odds ratio of 1.0 by definition, with no confidence interval.
‡*Sexually active* was defined as engaging in sexual intercourse ≥3 times per month.

a history of hysterectomy was associated with a 20% decrease in the odds of having a pH of 5.0 to 5.5 (Table IV).

Ten of eleven women with diagnosed cervical cancer had a vaginal pH of 5.0 to 5.5. The age range of these women was 21 to 73 years and their mean age was similar to that of women with normal cytologic results (40.0 ± 14.4 among 11 women with cancer versus 42.1 ± 15.4 among 8211 women with normal cytologic results; *t* test, $P = .65$). Thus age differences are unlikely to explain the relationship between vaginal pH and cervical cancer. Other cervical lesions (equivocal, low-grade squamous intraepithelial lesions, and high-grade squamous intraepithelial lesions) were not associated with changes in vaginal pH, nor was HPV deoxyribonucleic acid positively associated with pH. Moreover, among the HPV-bearing women vaginal pH was not associated with cervical diagnosis after adjustment for age and other predictors of vaginal pH (data not shown).

The use of vaginal douching was assessed among a subset of women (n = 2136) who were referred for a colposcopic examination and received a supplemental questionnaire. This subset of women included all women with cancer or high-grade squamous intraepithelial lesions (n = 141), 95% of women with low-grade squamous intraepithelial lesions or a cytologic evaluation of atypical squamous cells of unknown significance (n = 770), 96% of women with equivocal cytologic findings but normal colposcopic appearance (n = 711), and a 5% random sample of all women enrolled (n = 515 excluding overlap with other groups). The associations with vaginal pH in this subset of women were similar to the associations observed among women not referred for colposcopy. We did not observe important differences in the percentage of women with vaginal pH 5.0 to 5.5 according to a history of use of vaginal douche preparations made with water and vinegar or other types of vaginal douche preparations. After adjustment for age, however, the past use of vaginal douches made with water and vinegar was associated with a 30% decrease in the odds of having an elevated pH of 5.0 to 5.5 (odds ratio 0.7, 95% 0.6-1.0). Further adjustment by other variables did not change this relationship. Increased duration of vaginal douching was not related to vaginal pH (data not shown).

In addition to the reported odds ratios for combined pH values of 5.0 to 5.5, we also estimated separately the odds ratios for values of 5.0 and 5.5. The magnitude of

Table III. Adjusted odds ratios for association between vaginal pH from 5.0 to 5.5 and selected variables among premenopausal women

	Vaginal pH 4.0-4.5		*Vaginal pH 5.0-5.5*			
	No.	*%*	*No.*	*%*	*Odds ratio**	*95% confidence interval*
Time since last menstrual period						
1-5 d (menstruation)	373	58.2	268	41.8	2.4	2.0-2.9
6-14 d (proliferative phase)	1584	72.6	598	27.4	1.2	1.1-1.4
15-30 d (secretory phase)	1931	76.9	579	23.1	1.0	—†
31-60 d‡	268	70.5	112	29.5	1.3	1.1-1.4
>60 d‡	343	72.7	129	27.3	1.3	1.0-1.6
Barrier contraceptive methods used in previous 1 mo						
No	3786	71.7	1495	28.3	1.0	—†
Yes	713	78.9	191	21.1	0.8	0.6-0.9
Intrauterine contraceptive device in previous 1 mo						
No	4159	73.3	1516	26.7	1.0	—†
Yes	340	66.7	170	33.3	1.4	1.2-1.8

*Adjusted for age, marital status, sexual activity in previous year, and history of yeast infection. Derived from complete data on 6185 premenopausal women.
†Reference category; odds ratio of 1.0 by definition, with no confidence interval.
‡Reasons for delay in menstrual period were injected hormone contraceptive use, lactation, and others.

Table IV. Adjusted odds ratios for association between vaginal pH from 5.0 to 5.5 and selected variables among postmenopausal women

	Vaginal pH 4.0-4.5		*Vaginal pH 5.0-5.5*			
	No.	*%*	*No.*	*%*	*Odds ratio**	*95% confidence interval*
Time since last menstrual period†‡						
≤5 y	378	48.7	398	51.3	1.0	—§
6-10 y	194	38.5	310	61.5	1.3	1.0-1.7
11-20 y	273	32.7	562	67.3	1.4	1.1-1.8
>20 y	172	23.4	562	76.6	1.5	1.0-2.2
History of hysterectomy						
No	745	33.2	1497	66.8	1.0	—§
Yes	275	44.6	342	55.4	0.8	0.7-1.0

*Derived from complete data on 2849 postmenopausal women.
†Defined as current age minus age at last menstrual period.
‡Adjusted for age, marital status, sexual activity in previous year, and history of yeast infection.
§Reference category; odds ratio of 1.0 by definition, with no confidence interval.

the association between vaginal pH and its predictors did not substantially vary between women with pH values of 5.0 and 5.5.

Comment

As reported in previous studies,[2, 7] our data indicate that vaginal pH is higher in postmenopausal women than in women still having menstrual periods. In addition, aging is strongly associated with increasing vaginal pH in both premenopausal and postmenopausal women. The observed increase in vaginal pH among premenopausal women might be explained by gradual changes in cycle length and hormone levels occurring during the menopausal transition. Prospective studies have shown that the total cycle length starts to decrease among women at around 40 years of age, becoming irregular during the transition.[15] The decrease in cycle length has been attributed to a shorter follicular phase, accompanied by an increase in serum follicle-stimulating hormone levels and a decrease in serum estradiol levels throughout the cycle.[16, 17] Thus vaginal pH could be a marker of hormonal changes among perimenopausal women.

Our results indicate that vaginal pH values during menstruation (days 1-5) and the proliferative phase of the menstrual cycle tend to be higher than values measured during the secretory phase, with values highest during menstruation. These results are in line with previous observations that pH values are the highest during menstruation, peaking at 7.0 during maximal flow (days 2-3) because of the blood pH. This increase is followed by a sharp drop in pH to 4.0 to 4.5 during the next few days. Vaginal pH remains low until about day 21, when there again is a steady increase in pH at onset of menses.[3]

Among postmenopausal women aging was associated with a continuous increase in pH until late in life, and for a given age a longer time since menopause was associated

with a higher pH. Plasma concentrations of follicle-stimulating hormone and luteinizing hormone are consistently elevated in postmenopausal women with respect to those in premenopausal women as a response to the decreased production of estrogens by the ovary.[18] A recent study reported high vaginal pH values associated with high levels of follicle-stimulating hormone and low levels of estradiol in postmenopausal women.[7] Levels of follicle-stimulating hormone and luteinizing hormone in postmenopausal women have not been seen to change with age,[19] however, and the decrease in estrogen levels after menopause appears to stabilize within a few years.[20, 21] Similarly, levels of progestins do not seem to change with increasing age.[21] On the other hand, plasma levels of some androgens decrease with aging.[21, 22] Thus it is unclear whether changes in hormone levels could explain the observed progressive and lifelong rise of vaginal pH with age. Other age-related factors could exist, such as gradual vaginal atrophy or decades-long changes in the vaginal flora.

A vaginal pH value of 4.5 to 4.7 without respect to the age of the woman is commonly used as 1 of the 4 clinical criteria for the diagnosis of bacterial vaginosis.[23] Failure to take the age-pH relationship into account could partially explain why bacterial vaginosis is more commonly diagnosed in older women than in younger women.[24] Moreover, when compared with the Gram stain criteria for bacterial vaginosis,[25] the vaginal pH criterion has good sensitivity but poor specificity; that is, the use of a vaginal pH of >4.5 as a criterion for bacterial vaginosis generates a large percentage of false-positive results.[23, 24] Taking the age-pH relationship into account might reduce this number of false-positive results.

After menopause sexually active women had a lower odds of having an increased pH than did sexually inactive women (odds ratio 0.7, 95% confidence interval 0.6-0.9). This result is consistent with a study among 34 postmenopausal women that showed that those who were sexually active had a mean pH value lower than those who were sexually inactive.[9] Higher frequencies of sexual intercourse and self-stimulation among postmenopausal women have also been shown to be associated with a lesser degree of vaginal atrophy.[8] The fact that in our population sexual activity but not frequency of sexual intercourse was associated with vaginal pH is difficult to explain. Sexual inactivity among postmenopausal women could be related to the discomfort of vaginal atrophy, which is associated in turn with an increased pH.

Our study has some noteworthy limitations. First, vaginal pH was assessed with paper strips that measure pH in increments of 0.5 units. Although the crudity of the pH measurement may have masked some existing associations, we believe that this measurement was appropriate for practical purposes since it is likely to be used in epidemiologic studies and clinical examinations. Second, we used self-report of absence of menstrual periods as a surrogate for menopause. Self-report is likely to be a good surrogate of menopause among women ≥40 years old, but it may misclassify relatively few women at younger ages who report not having menstrual periods but have functional ovaries. If this type of misclassification is not related to the vaginal pH measurements, the result of the misclassification would be to underestimate the impact of menopause (or lack of ovarian function) on vaginal pH. Third, we were unable to consider the effects of current vaginal conditions responsible for increases in vaginal pH, such as bacterial vaginosis and trichomoniasis. Thus the increase in vaginal pH observed with increasing age could be strengthened by taking into account conditions[26] as their prevalence decreases with age. Finally, we were also unable to explore the effects of current or recent pregnancy on vaginal pH because pregnant women and women within 3 months after the end of pregnancy were excluded from the study and information on time since last pregnancy was not collected.

Unexpectedly, vaginal douching was associated with a slightly lower vaginal pH, with no apparent effect of douching duration. A history of sexually transmitted diseases, current HPV infection, or intraepithelial lesions was not associated with vaginal pH. The observed elevation of pH in women with cancer was almost certainly a secondary effect of tumor diathesis. On the basis of our results, vaginal pH could be a marker of age-related hormonal changes; however it is unlikely to be a biologic marker of hygiene or vaginal infection in epidemiologic studies of cervical neoplasia. We will continue to study the use of vaginal pH as part of a more complex assessment of inflammation in the natural history of cervical neoplasia.

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